

Applicant : Hagan Bayley et al.
Serial No. : 09/784,985
Filed : February 15, 2001
Page : 2 of 8

Attorney's Docket No.: 07917-062002 / UMMC 98-03F

Amendments to the Claims:

This listing of claims replaces all prior versions and listings of claims in the application:

Listing of Claims:

1 to 4 (Canceled)

¹
~~5.~~ (Original) A staphylococcal alpha hemolysin (α HL) polypeptide comprising at least two non-consecutive heterologous amino acids in a stem domain of said polypeptide, wherein each of said heterologous amino acids binds a metal.

¹
~~6.~~ (Original) The polypeptide of claim ~~5~~, wherein said amino acids occupy two or more of the following positions of SEQ ID NO: 1: 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147 or 149.

³
~~7.~~ (Original) The polypeptide of claim ~~5~~, wherein said amino acids occupy two or more of the following positions of SEQ ID NO: 1: 110, 112, 114, 116, 118, 120, 122, 124, 126, 128, 130, 132, 134, 136, 138, 140, 142, 144, 146, 148.

⁴
~~8.~~ (Original) The polypeptide of claim ~~5~~, wherein said polypeptide comprises at least three non-consecutive heterologous amino acids in the stem domain of said polypeptide.

⁵
~~9.~~ (Original) The polypeptide of claim ~~5~~, wherein said polypeptide comprises at least 4 non-consecutive heterologous amino acids in the stem domain of said polypeptide.

⁶
~~10.~~ (Original) The polypeptide of claim ~~9~~, wherein said amino acids occupy positions 123, 125, 133, and 135 of SEQ ID NO: 1.

Applicant : Hagan Bayley et al.
Serial No. : 09/784,985
Filed : February 15, 2001
Page : 3 of 8

Attorney's Docket No.: 07917-062002 / UMMC 98-03F

⁷
11. (Original) The polypeptide of claim ⁶~~10~~, wherein said polypeptide is 4H.

⁸
~~12~~. (Presently Amended) A mutant staphylococcal alpha hemolysin polypeptide comprising a heterologous amino acid, wherein the heterologous amino acid binds an analyte and wherein the polypeptide assembles into a heteroheptameric pore assembly in the presence of a plurality of wild type staphylococcal alpha hemolysin polypeptides ~~The polypeptide of claim 1,~~ and wherein said the amino acid is selected from the group consisting of Ser, Thr, Met, Trp, and Tyr.

⁹
~~13~~. (Presently Amended) A mutant staphylococcal alpha hemolysin polypeptide comprising a heterologous amino acid, wherein the heterologous amino acid binds an analyte and wherein the polypeptide assembles into a heteroheptameric pore assembly in the presence of a plurality of wild type staphylococcal alpha hemolysin polypeptides ~~The polypeptide of claim 12,~~ and wherein said the amino acid is selected from the group consisting of Glu, Asp, Cys, His.

¹⁰
~~14~~. (Original) The polypeptide of claim ⁹~~13~~, wherein said amino acid is His.

¹¹
~~15~~. (Original) A staphylococcal alpha hemolysin (α HL) polypeptide comprising at least two non-consecutive heterologous amino acids in a stem domain of said polypeptide, wherein each of said heterologous amino acids binds an organic molecule.

¹²
~~16~~. (Original) The polypeptide of claim ¹¹~~15~~, wherein said organic molecule is an explosive.

¹⁴
~~17~~. (Original) The polypeptide of claim ¹¹~~15~~, wherein said amino acids occupy two or more of the following positions of SEQ ID NO: 1: 111, 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147 or 149.

Applicant : Hagan Bayley et al.
Serial No. : 09/784,985
Filed : February 15, 2001
Page : 4 of 8

Attorney's Docket No.: 07917-062002 / UMMC 98-03F

¹³
18. (Original) The polypeptide of claim ¹²16, wherein said polypeptide is 123W/125W.

¹⁵
19. (Presently Amended) The polypeptide of claim ¹¹15-1, wherein said polypeptide further comprises a third second heterologous amino acid at a site distant from said stem domain.

¹⁶
20. (Presently Amended) The polypeptide of claim ¹⁵19; wherein said third second heterologous amino acid is a Cys residue at position 292 of SEQ ID NO: 1.

21 to 25 (Canceled)

¹⁷
26. (Presently Amended) A heptomeric pore assembly comprising a mutated staphylococcal α HL polypeptide (MUT), wherein the MUT ~~The pore assembly of claim 21, wherein said analyte-binding α HL polypeptide is 123W/125W.~~

27 to 29 (Canceled)

¹⁸
30. (Presently Amended) A digital biosensor device comprising a heptomeric pore assembly comprising a mutated staphylococcal α HL polypeptide (MUT), wherein the MUT is an analyte-binding α HL polypeptide comprising ~~The device of claim 29, wherein said analyte-binding α HL polypeptide comprises at least two nonconsecutive heterologous amino acids in the stem domain of the polypeptide, wherein each of said the heterologous amino acids binds a metal.~~

²²
31. (Presently Amended) A digital biosensor device comprising a heptomeric pore assembly comprising a mutated staphylococcal α HL polypeptide (MUT), wherein the MUT is an analyte-binding α HL polypeptide comprising ~~The device of claim 29, wherein said analyte-~~

Applicant : Hagan Bayley et al.
Serial No. : 09/784,985
Filed : February 15, 2001
Page : 5 of 8

Attorney's Docket No.: 07917-062002 / UMMC 98-03F

~~binding α HL polypeptide~~ comprises a chelating molecule in the stem domain of ~~said the~~ polypeptide.

¹⁹
~~32~~. (Presently Amended) The device of claim ¹⁸~~30~~ 29, wherein said device detects binding of a metal ion to said analyte-binding α HL polypeptide.

²⁰
~~33~~. (Original) The device of claim ¹⁹~~32~~, wherein said device detects a single channel current.

²¹
~~34~~. (Original) The device of claim ¹⁹~~32~~, wherein said device detects a current through two or more channels

35 to 45 (Canceled)

²³
~~46~~. (New) The device of claim ²²~~31~~, wherein said device detects binding of a metal ion to said analyte-binding α HL polypeptide.

²⁴
~~47~~. (New) The device of claim ²³~~46~~, wherein said device detects a single channel current.

²⁵
~~48~~. (New) The device of claim ²³~~46~~, wherein said device detects a current through two or more channels